THE ORIGIN AND DEVELOPMENT OF LAMELLAE IN *AGARICUS CAMPESTRIS* AND IN CERTAIN SPECIES OF COPRINUS[1]

MICHAEL LEVINE

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INTRODUCTION

The history of the study of the development of the Basidiomycetes dates back to the earlier part of the nineteenth century. Nees von Esenbeck (1816) should be credited with the description of the origin and development of these plants based on a philosophical conception of how they should develop rather than how they actually develop. Yet many writers, such as Dutrochet (1837), Trog (1837), and others, coupled these notions of Nees von Esenbeck with some actual observation. They were able to distinguish the two essential stages in the life of the mushroom, namely, the vegetative and the reproductive.

Toward the middle of the last century, Schmitz (1842)[2] first described an annular gill cavity separating pileus and stipe in several species of Basidiomycetes and was the first to recognize the veil or cortina. Schmitz conceived a method of development for all pileate fungi in which the organ nearest the substratum in the mature form is the structure first to develop, so that the mycelium is developed first, the stipe second, and the pileus next, the hymenophore being formed last.

Bonorden (1851), working about the same time, was the first to describe the structure of the volva and the manner in which dehiscence is effected. He contributed nothing to the origin of the parts of the plant.

Hoffmann's (1856) observation of the development of the carpophore lies at the basis of many current accounts of the methods of formation of the pileus and hymenium. He described the young buttons of *Agaricus campestris* as small spheres which elongate owing to the growth of the interior cells perpendicularly upward. The terminal cells now grow out laterally and then turn abruptly downward; the ends of these hyphae form the primordia of the lamellae. In 1860 and 1861 Hoffmann described the development of seventeen different species of higher Basidiomycetes. He contends that the fungus first appears in the form of a small white sphere in which a deeply colored central portion of the pileus rudiment

[1] The author wishes to express his indebtedness to the Carnegie Institution of Washington for assistance with the publication of the illustrations.

[2] Schmitz studied *Coprinus niveus, Cantharellus sinuosus, C. tubeaformis, Agaricus Bulliardi,* and *Hydnum imbricatum.*

appears. The formation of the gills resembles that of the other angiocarpous forms he described earlier.

De Bary's (1866) work followed soon after Hoffmann's. He described three species[3] of agarics in which he claims to have observed that the young carpophore begins as a mass of delicate and densely interwoven hyphae. Soon this small ball of hyphae becomes divided into two parts by the development of a horizontal annular gill cavity which in median longitudinal section appears as two openings in the upper and inner portion of the undifferentiated hyphal mass. The region lying above a horizontal plane through the gill cavity forms the pileus, while the part below forms the stipe. The layer of hyphae directly above the gill chamber grows into it and forms the lamellae. De Bary's figures of *Coprinus micaceus* in young stages clearly shows the edges of the gills in contact with the stipe, though he does not emphasize their connection with it.

R. Hartig (1874) claims that in *Agaricus* (*Armillaria*) *melleus* the lower surface of the pileus forms an exposed hymenium. A vigorous downward growth of the hyphae sets in from the upper surface and margin of the pileus and a corresponding upward growth of the superficial hyphae of the stipe, thus forming a weft of hyphae or a veil hiding from view the hymenium.

Brefeld (1877) claimed that the carpophore *Anlage* is a hyphal cell that gives rise to a number of branches which intertwine, forming a small mass of coiled hyphae. This mass increases in size, and internal differentiation ensues. The whole carpophore is covered by a loose layer of globular cells which Brefeld held is morphologically equivalent to the volva of the Amanitas and differs only in that in the latter genus the structure is more compact. Brefeld held that the lamellae arise as compact bundles of parallel hyphae each of which has apical growth. Numerous branches are produced which turn to the right and left to form the hymenium. The growth of the lamellae results finally in the adhesion of their edges to the surface of the stipe.

Fayod (1889) contributed his results of the study of a long series of agarics in which he denies Hoffmann's conception of the method of development of the pileus and hymenium. This author maintains that in the upper portion of the spherical button a layer of dense hyphae is differentiated which has the form of an inverted bowl that he calls the *couche piléogène*.

A great number of workers who have studied the sexuality of the Basidiomycetes since the nineteenth century have reported incidentally various phases of the development of these plants, but have not contributed anything of importance to the question of the origin of the gills (see Levine, 1913).

From the time of Fayod nothing was done until the appearance of Atkinson's (1906) work. This took up the question and the manner of origin and development of the carpophore of *Agaricus campestris* vars.

[3] *Agaricus campestris*, *A. praecox*, and *Coprinus micaceus*.

Columbia and Alaska. Atkinson claims that the gill rudiments appear first in a longitudinal median section of the young carpophore as two deeply stained areas. These areas represent the cross section of a heavily stained horizontal ring which is the primordium of the hymenium, composed of hyphae which have a very dense protoplasm. The hyphae below this structure rupture, and an annular hollow gill cavity is formed. The lamellae are formed by a downward growth of hyphae from the hymenium primordium into the gill chamber. These observations were confirmed by Atkinson on several other species of Agaricus.

The author of the present paper pointed out (1914) that the origin of the gills in agarics was not yet clearly understood. It was then shown that in the development of the lamellae of *Coprinus micaceus*, the young hymenophore arises at or near the lower surface of the pileus primordium in a manner similar to that described for a number of agarics by Hoffmann (1860), and that it appears in a vertical section of a young carpophore as two densely staining areas of palisade cells, placed to the left and right above the center of the young carpophore.

I pointed out further that the development of this annular primordium of the hymenophore consists in further elongation and, at the same time, specific orientation of these palisade cells. This results in the formation of a series of arched interhyphal spaces or primordia of gill chambers, while the adjacent palisades become the gill rudiments. The point which was emphasized was that the hymenial elements are formed over the arched surface of the interhyphal spaces, rather than on the edges of gill ridges extending downward into an annular gill cavity. In other words, the gill rudiments at this stage are continuous above and below with the fundamental tissues of the stipe and pileus. While De Bary (1887) figured this condition in the development of young buttons of Coprinus, he failed to point out its significance. The report of my observations on *C. micaceus* appeared about the same time as Atkinson's (1914*c*) description of the development of the carpophore of *Amanitopsis vaginata*. No primitive annular gill cavity is formed in this species. The young lamellae extend from the pileus fundament to the stipe region. In this respect the development of Amanitopsis species is like that of Amanita described by De Bary and Brefeld and like that of *Coprinus micaceus* described by me, but, according to Atkinson, is unlike the development of all other agarics. The growth at the margin of the pileus primordium is accompanied by a continuation of the differentiation in the fundamental tissue.

This development in Amanitopsis is identical with the stages found in plants of *Coprinus micaceus* and of other species of Coprinus that I have studied. But for the bulbous base of the stipe region and the thickened volva, sections of Amanitopsis and of Coprinus resemble each other very closely [see figures 83, 92, and 93, Plate XXXIV, and compare them with Atkinson's (1914*c*) figures 10, 13, and 14].

Atkinson reports a primitive annular gill cavity for *Agaricus campestris* (1906), *A. arvensis*, and *A. comtulus* (1914*a*), and *Lepiota clypeolaria* (1914*b*). In *L. clypeolaria* the hymenophore primordium is described as at first smooth. Later, folds are formed which develop into gills. His sections of this material are conspicuous and particularly interesting for the absence of a palisade layer at the time the annular gill cavity is formed (1914*b*, figs. 7–9). In this respect, *L. clypeolaria* is made to differ from all other forms he describes. This appearance is clearly due to the abnormal rupturing of the fundamental tissue below the hymenophore as I am describing it later in this paper.

Prior to 1915, Atkinson classifies the agarics, in which the lamellae arise endogenously, into two groups: those forms in which there is a so-called well-marked annular gill cavity formed below the primordium of the hymenium, as in *A. campestris*, etc., and those in which there is no general gill cavity but rather a series of cavities as in Amanita, Amanitopsis, and in Coprinus described by me. For *Agaricus Rodmani*, Atkinson (1915) finds a new type of annular gill cavity. In this case the general annular gill cavity is interrupted by strands of fundamental tissue which extend from the hymenophore primordium to the stipe region below. These strands are conspicuously attached and are continuous with the tissues of the stipe and pileus and remain attached to the gill fundaments until well-differentiated lamellae are formed. Atkinson is unable to give any adequate explanation of these conditions on his theory that the gill cavity should be annular from the first.

In 1916, Atkinson reports finding a weak prelamellar gill cavity in *Coprinus micaceus* and *C. atramentarius*. In *C. comatus* a well-marked, "strong" annular prelamellar gill cavity is reported. In the same year, Atkinson (1916*b*) reports in *Lepiota cristata* a "weak" annular prelamellar cavity, and in *L. seminuda* a very strongly developed annular gill cavity.

Douglas (1916) studied five species of Cortinarius[4] to determine the origin of lamellae and also the development of the universal veil, but gives no data as to the origin of the gill cavity.

Sawyer (1917*a*) found in *Pholiota squamosa*, *P. flammans*, and *P. adiposa* the appearance of "weak" annular prelamellar gill cavities, although the degree of "weakness" varies in the different individuals. The gills which he finds partially attached to the fundamental tissue become free very late, just before the gills are exposed by the rupturing of the partial veil. Sawyer fails to show the critical stages in the development of the gill salients. His preparations are made of plants too old, and in many cases probably dead, before fixation, although he failed to recognize this fact. His work on *Cortinarius pholideus* (1917*b*) shows nothing further as to the origin of the gill cavity.

Walker (1919) reports on the development of *Pluteus admirabilis* and

[4] *Cortinarius distans*, *C. cinnamomeus*, *C. armillatus*, *C. lilacinus*, and *C. infractus*.

Tubaria furfuracea. Although Miss Walker was unable to find stages early in the development of *Pluteus admirabilis*, she contends that in this form the primordium of the hymenophore is exogenous in origin and later becomes endogenous, a claim similar to that made by R. Hartig in 1874 for *Armillaria mellea* which was later corrected by Atkinson (1914*d*).

The studies reported by Atkinson and his students were made on material collected in the field. Specimens were fixed generally with chrom-acetic and picric-acid solutions. In no case so far as reported was the history known of the group of buttons studied. The assumed ages were generally based on size rather than on actual knowledge of the time of appearance of the button.

Adams (1918), in studying the development of *Schizophyllum commune* grown in artificial culture media as well as of plants collected in the field, found, contrary to all observations previously made by other investigators on this form, that the hymenium primordium of this plant arises endogenously. He shows clearly that there is no general annular gill cavity formed into which the young gills develop by downward growth, but that a series of successively formed endogenous gill chambers are produced which become ultimately the spaces between each pair of adjacent gills. These results agree entirely with my observation on the method of origin of the gill cavities in Coprinus. Adams concludes that the gills are essentially hymenium-bearing plates between schizogenously arising gill cavities.

Material and Methods

Since 1913 I have been studying cytologically the development of a number of species of agarics grown in controlled cultures. I have worked especially with *Coprinus ephemerus*, *C. stercorarius*, and *Agaricus campestris* var. *Bohemia* and a white variety possibly *Columbia*, spawn of which was bought from an American spawn producer. The species of Coprinus studied were selected, first, for the ease with which they may be grown under laboratory conditions on their natural substratum as well as on agar; second, for the rapidity with which they grow and develop from spore to mature carpophore; and third, because of the relatively small number of gills. Spores of *Coprinus ephemerus* and of *C. stercorarius* were sown on a variety of agar media. The most favorable was made of a horse-dung decoction. Within seven to ten days after inoculation a great number of young carpophores made their appearance on this medium. These cultures grow best at a temperature of from 20°–25° C. Carpophores were produced more abundantly during the winter and spring months than during the summer. They were more nearly normal in appearance when grown in semi-darkness.

It was found impracticable to grow *Agaricus campestris* in sufficient quantity *in vitro*. My cultures were grown in a mushroom cellar in the greenhouses of Columbia University. The space available, however, was

relatively small, and for comparison it was found desirable to study the general yield, weight, and size of the mushroom as grown in commercial mushroom houses in the vicinity of New York City. The mushroom clusters as they appear in the beds from the very youngest stages were plotted on quadrille paper, and each individual in a cluster was numbered and definitely located so that its whole history could be followed. The horizontal diameter of the apical region of the button was measured from day to day until it reached maturity, *i.e.*, until it became fully expanded or failed to increase in size, turned brown, and died. In this manner the growth curve and fate of each plant were carefully studied. The percentage of buttons that developed normally, the relative rate of growth, and the size and weight of the plant were determined and recorded. The data thus obtained soon enabled me to predict after two days in the case of any particular button whether or not it would reach maturity. The results of these studies I shall report elsewhere; here I wish to report on the gill development as shown in the case of these buttons of known age and normal development.

Fixations were made of white buttons that had just made their appearance on the surfaces of the beds, and of all later stages of normal buttons. Plants also that failed to show any increase in size and those that turned brown were fixed in a great variety of fixing solutions at different intervals. Agar-grown specimens of *Coprinus ephemerus* and *C. stercorarius* were fixed in a great number of different fixing solutions, the best of which was a dilute Flemming's weak solution. The plants were imbedded in paraffin and sections 5 to 25μ were made and stained. Sections of fixed buttons of *Agaricus campestris* were also compared with those of living material.

Agaricus campestris

The accepted facts as I have described them for *Coprinus micaceus* relative to the development of the simplest carpophore of the so-called endogenous types are: First, that the young, undifferentiated carpophore becomes divided into pileus and stipe rudiments. Second, the primordia of the hymenium arise as a circle or whorl of palisade pockets at or near the lower surface of the pilear rudiment, so that in a longitudinal median section of the young fruit body portions of this series appear as two densely staining narrow areas of hyphae on both sides of the button slightly above the center. Third, the growth of the hyphae of the pileus rudiment is marginal, that is, the youngest portion of the pileus is at its margin. I have pointed out for *C. micaceus* that there is no general annular gill cavity as maintained by Hoffmann, Atkinson, and others for a number of agarics, into which radially arranged plates of hyphae, primordia of the lamellae, grow, but that the palisade cells orient themselves on both sides of vertically arranged interlamellar spaces so as to form a series of gill cavities and gill

Anlagen. Where the ends of the palisade hyphae meet, the interlamellar space, the gill cavity *Anlage*, is at first tightly closed and does not appear as an open interhyphal space even with high magnification. The hyphae between two such adjacently formed palisade layers constitute the *Anlage* of the trama. These hyphae can be traced back into the rudiment of the pileus and into that of the stipe.

The results I obtained in *Agaricus campestris* are similar to those obtained with *Coprinus micaceus*. Atkinson and his students have noted that the fixation of larger types of gill fungi is exceedingly difficult. The large continuous annular gill cavity which they describe is, as I find, an artefact due to poor fixation. I have studied the development of *Agaricus campestris* from material grown under observation in mushroom beds and have been able to follow the development, growth rate, etc., of these plants from the time they appeared on the surface of the bed to the time they are fully expanded. I have compared sections of fresh and fixed material at all stages. It is at once discovered in such studies that not all the buttons which appear in young clusters are destined to reach maturity, as noted by Duggar (1915) and others. Studies of growth rate, etc., enabled me to predict which buttons are going to develop and which are not. Generally, after 72 to 96 hours after the appearance of the buttons on the substratum those that are destined to develop can be distinguished from those that are not. At this time there is no conspicuous difference in their appearance, but only in their ability to grow as shown by their previous rate of growth. Later on, those that do not develop turn brown.

Young plants that showed constant increases in size by actual measurements were sectioned free-hand and studied quickly thereafter under the microscope. The sections showed, in the young and late stages of the development of the palisade pockets and gill primordia, no annular gill cavity. The hymenophore primordia and the rudimentary gills shown in Plate XXVIII, figures 1–16, are firmly attached to the fundamental tissue of the stipe below, just as they are in *Coprinus micaceus*, *C. ephemerus*, and *C. stercorarius* mentioned below. On the other hand, specimens similar as to age and size fixed in the various commonly used fixing solutions showed within twenty minutes after fixation two large holes placed right and left of the stipe (figs. 35–51), just below the young pileus and corresponding exactly to the annular gill cavity described by Atkinson. It should be added that great care must be taken in making these sections since the forming palisade pockets weaken in the tissue connection between pileus and stipe. Young buttons that showed no sign of developing normally and those that turned brown were also studied in free-hand sections. These showed conditions similar to that in the unfixed living plants. There was no annular gill cavity, and the gill *Anlagen* were in connection with the fundamental tissue below the pilear region (figs. 17–22). When the apparently dead carpophores were fixed in chrom-acetic and various other

fixing agents they showed no annular gill cavities and did not differ from the unfixed living or unfixed dead specimens (figs. 23–28).

It appears that the already inert cells are less likely to shrink in fixation than are those of the living and growing plants. For this reason fixed material of dead plants was studied, the living plants shrinking too much to give a true picture of the conditions even with the fixing agent generally conceded to be the best for fungi. Figures 29–51 represent free-hand sections of living buttons approximately of the size and age in which gill primordia are developing after having been fixed for 24 hours in the following fixing agents: chrom-acetic, Bouin's picro-formol, picro-acetic Carnoy's, Gilson's, Kaiser's, Flemming's strong, medium, and weak, Juel's, and Merkel's. It appears from these figures that the chrom-acetic, picric acid, and Flemming's strong solutions produce the greatest amount of tearing of the fundamental tissue just below the lamellar region.

Flemming's weak solution produced the least shrinkage of all fixing agents (figs. 29–34), but still sufficient shrinkage to vitiate the results. For this reason it was found that detailed histological studies were best made from fixed dead carpophores simultaneously with a study of free-hand sections of fresh ones. The dead carpophores after being fixed were carried through the alcohols to paraffin with the greatest care and then sectioned.

As to the early development of Agaricus species my results from the fresh and fixed dead plants confirm those of De Bary (1887), Hoffmann (1860), and others. The development of the gill primordia is essentially similar to that in the Coprinus species I have described. The gill cavities originate separately and not as a continuous annular or ring-shaped opening. They arise as arched series of palisade cells which appear very early between the vertically arranged radial plates, the trama primordia, so as to form a series of wide archways, the rudimentary gill cavities. The tramal elements are in connection with the fundamental tissue of the stipe region below. In *Agaricus campestris* the rudimentary gills differ in shape from those of the Coprinus species I have studied, in that they are broader near the pileus and more or less wedge-shaped as they approach the fundamental tissue of the stipe region; so that, when shrinkage of the tissue occurs, one studying a tangential longitudinal section is given the impression that the gills grow downward into a large cavity.

Sections of fresh buttons together with prepared dead carpophores of approximately the same age and size show that the wedge-shaped gill primordia are attached to the fundamental tissue of the stipe region, and very often the gill cavity *Anlagen* are invaded by fundamental tissue from below giving a picture similar to those described by De Bary (1866), Brefeld (1877), and Atkinson (1914*c*) for Amanita and Amanitopsis species. These stages were reproduced satisfactorily only by photographic methods from carpophores that were dead and which were put into Flemming's weak solution, sectioned, and stained, although similar figures were obtained from free-hand sections of the living carpophores.

Figure 52, Plate XXIX, represents a longitudinal tangential section of a young button in which a number of gill *Anlagen* are shown. The margins of these young gills are in connection with the tissue below them, and in the center or oldest part of the section gill cavities are already formed. While this is a microphotograph of a plant that died very early in its development, similar stages were observed in the living buttons. In the unstained sections of young living buttons the gill chambers are wider near the stipe region. The palisade cells which cover the upper surface of the gill chambers are small cells which are not markedly oriented as we find them in the Coprinus species. The trama primordium is very difficult to distinguish in unstained material, yet it has been made out clearly to consist of a narrow band of tissue which is composed of cylindrical cells, which are continuous with the cells in the stipe below and in the pileus above. In the preparation of the dead plant as shown in the above-mentioned figure, the palisade cells do not stain strongly, but a thin, pale violet stain is present between the compact mass of cells of which it is composed. The trama walls stain heavily. The fundamental tissue below the gill cavities consists of a loose plectenchyma which occasionally shows a disintegrating nucleus.

In an older stage of a button, also slightly larger, shown in the fresh carpophores, the tramal elements are in close connection and continuous with the hyphae of the fundamental tissue of the stipe and pileus. The gill primordia are considerably larger, while the palisade cells are longer and have become more numerous but maintain the same shape. In a fixed button that had turned brown at the same age and size, shown in figure 53, the tramal elements are seen clearly in connection with the stipe and pilear tissue. The triangular arch of the gill cavity is covered by palisade cells which stain faintly. The highly granular appearance of the fundamental tissue below the gill rudiment is due to crystal deposits in the cells.

A median and tangential section of another dead carpophore (fig. 55), of the same size but fixed two days after the one shown in figure 53, shows that the palisade cells have largely disappeared and can only occasionally be recognized around the broad and deeply staining tramal tissue. The hyphae of the fundamental tissue are deeply stained also, and, as in the living carpophores of this age, long strands of hyphae can be traced directly into the trama of the young gill rudiments. In the tangential section shown in figure 54 a number of the gills to the right are disconnected from the fundamental tissue below them. This is due to the splitting and collapse of the fundamental tissue as indicated by the more compact plectenchyma of hyphae shown at *B*. This figure is comparable with the figures of Atkinson (1906, figs. 12, 19, 32–38) and Sawyer (1917*b*, fig. 37); these authors, however, failed to recognize that the material they were studying was dead when fixed as evidenced by the disappearance of the palisade cells and the apparent continuity of the tissue of the rudimentary gills

with the undamental tissues after fixing in picro-acetic or chrom-acetic acid, as my experiments show. They explain this structure by assuming that this continuity of tissue is merely contact of the young gill with the rudimentary stipe due to the force exerted on the fundamental tissue by the involute margin of the pi eus, bringing this tissue in close contact with the young gill.

Buttons that showed no increase in size and that had been on the surface of the substratum for 48 to 72 hours after they failed to show signs of growth, and fresh buttons of approximately the same age, showed stages in which the young gills were well formed. In those specimens that were already beginning to turn brown, the stretching and expansions of the tissues were retarded. These plants show but slight evidence of an involute margin (Pl. XXX, figs. 59, 60, 61), yet the fundamental tissue of the stipe region is continuous with the gills. In the sections shown in figures 59 and 61, the sagging of the tissue below the gills causes the formation of the deeply stained line which in a photograph tends to give one the impression of an artefact, but under the microscope hyphae can be traced from the fundamental tissue into the young gills. This sagging or collapse of the tissue is associated with loss of turgescence and necrosis. In all three cases the palisade cells are devoid of cytoplasm, and in figures 60 and 61 they seem to have broken down, giving a ragged appearance to the layer, which is generally even and smooth at this stage.

In a study of buttons of *Agaricus campestris* that have turned brown after they had been on the surface of the substratum for 96 hours without showing any signs of growth, we find conditions such as are represented in figures 56, 57, Plate XXIX, and figure 58, Plate XXX. The first of the series (fig. 56) represents a longitudinal tangential section toward the margin. This plant, as we can judge from the median section (fig. 58), has no marked involute margin and is similar to normal buttons of this size and age before death. There is no incurved margin, yet we see deeply stained tramal elements attached firmly to the fundamental tissue (fig. 56). Faint indications of the palisade cells bounding the gill cavities can be seen in this figure. Though these buttons are dead, these sections offer further evidence of the continuity of the gill elements with the pileus region above and with the fundamental tissue of the stipe below. In this preparation and in stages of living plants of the same age we find quite conclusive evidence that the palisade layer is not followed by a region toward the periphery of the hymenophore primordium which subtends an annular gill cavity, as maintained by Atkinson and his students, but that this region is closely in contact with the fundamental tissue below it, as shown here. As our sections approach the median we find that the gills have already become separated from the fundamental tissue, which evidently develops into the annulus. It is of interest to note in these sections near the stipe the furrows of the fundamental tissue which are well shown in figures 57 and 58 directly

below the margins of the young gills. Figure 57 represents a view near the stipe region, and we see the gills already torn away from the tissue below. This fundamental tissue below the gills has collapsed partly and has formed a thin, regular curve (in outline), deeply stained, which marks the upper surface of the annulus (*A*). The fine radial grooves which are noticed on the rings of annulate species and on the upper portions of the stipes of exannulate species are marks of this continuity which exists between the hyphae of the stipe and the tramal elements of the gills. Harper's (1913, 1914) excellent photographs of annulate and exannulate fungi bring out these facts clearly. The gills and the furrows seen in outline are approximately equal in number, and the shape of the margin of the gill seems to indicate that it is the counterpart of the furrow of the annulus.

It appears, then, from the study of both the living and the dead buttons of *Agaricus campestris* that the method of the development of the lamellae is essentially the same as in Coprinus species. The early stages in the formation of the gills in Agaricus show more clearly the relation of the tramal elements to the palisade cells and that they form bridges of hyphae between the pileus and stipe regions. The orientation of the palisade cells on both sides of the trama forms typical arches enclosing gill cavities. One apparent difference between the early gill formation in Coprinus and in Agaricus species is that in the latter the palisade cells which arise from the lower portion of the trama are at first shorter than those that arise higher up from the pilear region, thus giving the young gill a wedge shape in section, the gill cavity forming from the beginning a true arch. It becomes clear that, as seen in section, the arch of palisade cells is the unit of structure of the hymenial surface. This palisade tissue originates as a mere pocket which elongates radially with the marginal growth of the pileus. These palisade groups widen on both margins by the formation of new elements which bud out from the trama. These new elements face each other, and the interpalisade space is the forming gill cavity.

Among the normal plants of *Agaricus campestris* grown from pure spawn there often appeared a button which was at first indistinguishable from the other buttons, but which became characterized by the smallness of the pilear region. These plants never mature as far as I have observed. Sections of these buttons were made, and it was found that the basal portion of the plant consisted of an undifferentiated mass of hyphae the elements of which are very narrow and very much intertwined, taking in general a uniform stain. The apical portion, which was marked off by a superficial annular furrow, was found to consist of five zones of tissue (figs. 62–65, Pl. XXXI). The central part consists of a hemispherical mass of intertwining hyphae, recalling at once Fayod's *couche piléogène*. This structure stained heavily. The hyphae in the middle of the central region are very much entangled, as mentioned above. Toward the surface, the hyphae become radiate and blended with a zone of much more faintly staining

hyphae immediately above. This in turn is followed by another broad, densely staining area, and this is succeeded by two other layers also staining deeply but not so wide. The significance of these regions is not clear, but it appears that by differentiation of the tissue between the *couche piléogène* and the light-staining tissue above it, the reproductive tissue is formed. Figures 64 and 65 show a median longitudinal section of the pilear region in which differentiation has set in and a number of poroid gills have been formed. The tramal elements shown in the enlargement (fig. 65) take the stain heavily, but the palisade cells are too faint to register an impression on the photographic plate. Such appearances as this suggest the possible origin of the gilled fungi from a poroid type.

Coprinus ephemerus and C. stercorarius

The young carpophores of *Coprinus ephemerus* and *C. stercorarius* generally appear at the margin of the agar culture and seem to arise from a comparatively stout rhizomorph-like strand of hyphae. Very often a small piece of agar containing hyphae of *C. ephemerus* and *C. stercorarius* transferred to another culture medium produced small greyish sclerotia, as shown by Brefeld (1877). Mycelial growth then continues, radiating from the inoculum to the margin of the agar where the carpophores are formed. Not uncommonly, small carpophores arranged in a radial series arise from a rhizomorph close to the sclerotium, as shown in figure 66 (Pl. XXXII). Subsequently minute carpophores arise all over the agar, as shown in figures 67 and 68. Young carpophores were fixed in Flemming's weak solution diluted to one quarter strength. The earliest stages in the development of *C. ephemerus* resemble in the main the stages described for *C. micaceus* (Levine, 1914). The young, undifferentiated button, less than .1 mm. in diameter, consists of a very much entangled mass of hyphae. The carpophore stains uniformly, although the enveloping outer hyphae end in large globose cells having thick walls. These cells very often stain heavily with the gentian violet of the triple stain. The primordium of the pileus does not stain differentially as readily as does that of *C. micaceus*, and consequently it is not easily recognized at first. The primordium of the hymenium, however, takes the stain heavily and is the first structure to be noticed in the differentiation of the carpophore. It consists of a horizontal series of palisade pockets and appears in the upper third of the young button; in longitudinal median section it appears, as it has been often described for other species of agarics, as two densely stained areas to the right and left of a median vertical line. In *C. stercorarius* (fig. 85, Pl. XXXV), the young, undifferentiated carpophore consists of a weft of mycelium in which strands of parallel hyphae can be traced for a considerable distance, the entire mass forming a more or less ovoid body and so differing from the corresponding stage of *C. ephemerus* and *C. micaceus*. In this species, also, one may notice that the terminal cells of the hyphae

do not become globular at this stage, but that the tips seem to converge toward the apex of the small button. The tip cells are more densely filled with cytoplasm and take the stain more heavily. It is interesting to note that the carpophore shown in figure 85 arose below the surface of the agar, and it appears in this figure as if a number of hyphae were involved in the initial development of the plant. Sections of small knob-like projections, which give rise to carpophores and which appear on the stipes of older but attenuated specimens of *C. stercorarius*, consist at first of an undifferentiated mass of hyphae, as shown in figure 86. Here it may be observed that, while the cells of the old stipe are large and generally highly vacuolated, the cells from which the young carpophore seems to take its origin are small and stain heavily. This type of young carpophore is somewhat more compact in structure, and the base of the plant stains less strongly than the apical region. In *C. stercorarius*, unlike *C. ephemerus*, the first change in the undifferentiated carpophore consists in the formation of a pilear region which answers the description of Fayod's (1889) *couche piléogène* (fig. 87). It appears to be an inverted flattened hemispherical mass of tissue consisting of very much entangled, deep-staining hyphae. The stipe region at this stage also stains somewhat deeply, and between the two a layer of undifferentiated, delicately stained tissue remains. The apical terminal cells of this plant are thick-walled; they lose their cytoplasm and fail to stain. It is quite likely that it is these cells which form the flaky structures on the surface of the mature pileus later in the development of the plant.

In both *C. ephemerus* and *C. stercorarius*, these early stages are followed more gradually by the development of pockets of palisade-like hyphal tips immediately below the pilear region. In *C. ephemerus* the undifferentiated primordium of the hymenophore region gives rise to these pockets of palisade cells, no distinct pilear region having been formed; in *C. stercorarius* they are formed on the outer part of the lower surface of the pileus rudiment. Previous to this stage, and during the development of subsequent stages, there is no indication of a general annular gill cavity such as is described by Atkinson and his students for other species of agarics. As I have pointed out above, the matter of successful fixation is of prime importance at this stage of development. It appears that the hyphae that are continuous between the stipe fundament and pilear region, the future trama, are very easily ruptured by shrinkage in fixation.

Gill Cavities

The palisade pockets of hyphal cells arise primarily from the pilear region and appear in longitudinal sections in distinct groups with the downward-growing hyphal tips slightly converging toward a common center within the group. The hyphae on the boundaries of such groups, the future trama cells, are continuous, as noted above, between the hyphae of the pilear and stipe rudiments.

Figure 69, Plate XXXII, represents a longitudinal tangential section of a very young carpophore in which there is no annular gill cavity, but the palisaded cells are arranged quite obviously into at least four distinct groups with three faintly stained trama areas (*A*) between them which are continuous with the stipe and pileus rudiments. As we shall see later, where the tips of the oriented palisaded cells of each pocket meet, a gill cavity will be formed. The strictly median longitudinal sections of the young carpophores which lie in the plane of the future gill cavity are, it is obvious, less favorable for the correct understanding of the gill origin than longitudinal tangential sections. Figure 70 represents such a longitudinal median section of the same button shown in figure 69; the group of cells faintly stained at the right is a section of the area of ce ls which form the boundary between two adjacent clusters of oriented palisaded hyphal cells. The cluster of deeply stained cells to the left represents a section through the palisade cells. Where the tips of the cells meet we have a radial view of the beginning of the gill cavity. It is already clear in this carpophore that the palisaded cells are well established, and yet there is no evidence of an annular gill cavity.

Figure 71, Plate XXXIII, represents a longitudinal median section of a young carpophore a little older than the one previously described. Here, as in figure 70, we find the two characteristic patches of palisade cells to the right and left, slightly above the center of the carpophore, toward the margins of the young pileus. There is still no indication of an annular gill cavity. As our sections become more tangential (fig. 72) we notice the grouping of the palisaded cells (at *A*) and also the more parallel hyphae which lie between the groups of oriented cells and will form the trama. In a section more eccentric shown in figure 73, there are four distinct groups of palisade cells and three pronounced tramal regions between them (at *C*). Within each group of palisaded cells there are already faint indications of gill cavities which cannot merge into one annular gill cavity unless the tramal cell connections are torn. The primordium of the hymenium is oldest nearest the stipe. Toward the margin it becomes progressively younger. Figures 71, 72, and 73 show a point of interest in that the layer of terminal cells seems to form a distinct covering and appears to correspond to what Brefeld called a general veil. This layer has been torn away from the pilear region in the process of sectioning.

A somewhat older carpophore is shown in figure 74. The section is longitudinal and not quite median, through two groups of palisade cells with their small gill cavities in the earliest stages of appearance. More tangential sections of this carpophore (fig. 75) show four distinct groups of oriented palisaded cells (at *A*) with their rudimentary gill cavities and the intervening tramal cells. A distinct and conspicuous gill cavity is visible in this figure in the second group of palisade cells from the left side. In a section of the same plant still more tangentially placed and shown in

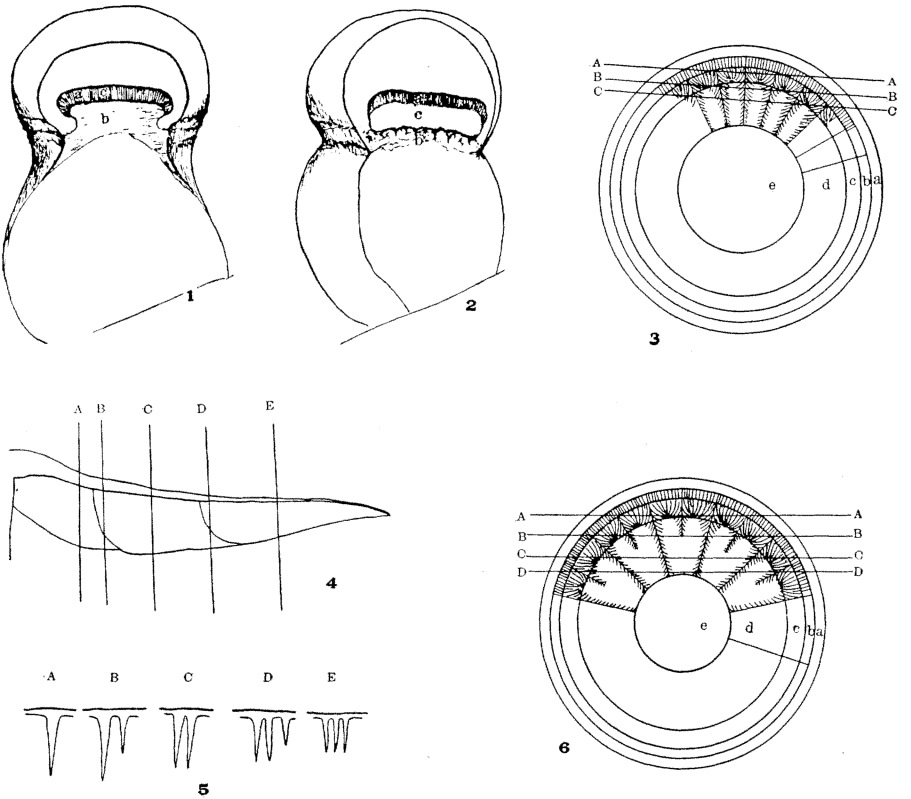
figure 76, we have three groups of palisaded hyphal cells and the gill cavities have entirely disappeared; that is, we have reached that portion of the hymenial primordium in which the palisaded cells while oriented have not as yet sufficiently separated to form a cavity. There is, of course, considerable fluctuating variability in the arrangement and the size of the gill chambers, and the sections are by no means exactly parallel to the longitudinal axis of the young carpophore. This axis, again, need not be a perfectly straight line. These facts will account for many apparent inconsistencies in the sections. It is, however, sufficiently clear from the figures that these rudimentary gill cavities arise at the region where the tips of the oriented palisaded cells meet. They become larger by an increase in the number of the palisade hyphae which seem to take their origin in the pileus and the tramal cells. The result is an arched rudimentary hymenial surface, under which the gill cavity becomes larger and larger as this hymenial surface increases and the walls of the two adjacent arches with their common trama become a single gill. In perspective we should have a series of these radially arranged arches and tramal plates, older and larger near the stipe and younger and smaller toward the margin. The tramal plates remain firmly attached to the stipe tissue below since their hyphae run through from pileus to stipe. The growth of the pileus rudiment is centrifugal, so that, while the rudimentary arch or gill cavity may be a wide opening near the stipe, it is just unfolding or opening at the margin of the pileus.

Tangential sections of older buttons are represented by figures 77, 78, and 79. In the middle of such a section the gill chambers are distinct openings (figs. 78, 79), taking the shape of Roman arches; toward the right and left they dwindle away, and we have only a pocket of hyphae with their tips pointing to a common center and no visible opening (fig. 77), In the last mentioned figure we find the two gill cavities still unopened or in a very rudimentary state. Each palisade cell contains two nuclei which stain well with Flemming's triple stain. There is no cytological indication at this stage that the older cells are to be found near the bases of the arches or rudimentary lamellae, as Buller (1909) suggested for the mature gills, and yet this observation does not indicate an inconsistency, for it is quite possible that the hymenium may mature and develop later from the margin of the pileus and outer edge of the gill upward, and be independent of the time of origin.

As we proceed still farther toward the center of the plant a large number of gill cavities appear, as shown in figure 79, which represents the maximum number that appear in a longitudinal tangential section of this button.

Figure 77 becomes more intelligible when studied in connection with diagram 3, which represents a top view of the carpophore in the earliest stages of development. The outermost space (*a*) between the two outer circles represents the region of the fundamental and undifferentiated tissue.

The next space toward the center (*b*) with small radial lines represents the smooth, unoriented palisaded layer of hyphal cells which is followed by a region (*c*) of oriented palisaded cells, and this in turn by the older portion



DIAGRAMS 1–6. 1. Longitudinal tangential section of a fresh carpophore showing the young lamellae (*a*), attached to the fundamental tissue (*b*), and forming a number of interlamellar spaces (*c*). 2. Longitudinal tangential section of a fresh carpophore after having been fixed from 6 to 24 hours. The young lamellae (*a*) are torn away from the fundamental tissue (*b*), leaving a wide annular gill cavity (*c*). 3. Top view of a carpophore in the earliest stages of development; the outermost space (*a*) represents fundamental and undifferentiated tissue; (*b*) smooth, unoriented, palisaded layer of hyphal cells; (*c*) oriented palisaded cells; (*d*) older portion of hymenophore primordium with gill cavities and gills; (*e*) stipe rudiment. 4. Radial section of pileus showing three gills of different lengths. 5. Various widths of gills shown by vertical cross sections made at various distances, *A*, *B*, *C*, etc., from the stipe. 6. Top view of carpophore showing secondary gills.

of the hymenophore primordium (*d*), that is, where distinct gill cavities and gills are already formed. The inner space represents a portion of the stipe rudiment (*e*). The line *AA* represents a vertical tangential section shown in figure 77, cutting through and exposing two groups of palisaded cells at the tips of which we find the youngest stage of two gill cavities. The line

BB represents the section shown in figure 78, where the two *Anlagen* of the gill cavities shown in figure 77 have opened and the two halves of the adjacent verticals of the arch form the gill; at the same time on both sides of these two gill cavities appear the eccentric views of two more gill cavities which are shown in figure 79, the approximate location of the section being represented in the diagram by the line *CC*.

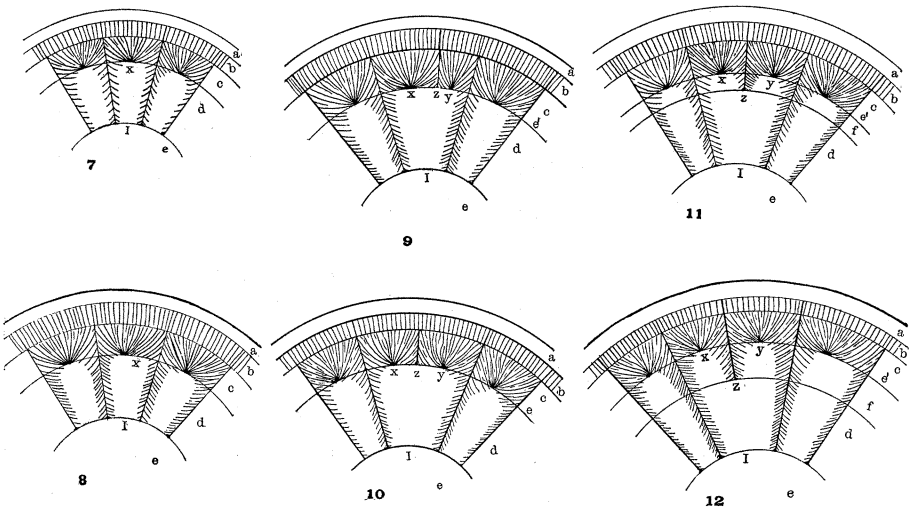
Secondary Gills

In buttons slightly older than those previously described we can study the origin of secondary gills. The origin of these gills is of special interest since the inner end of the gill often appears in a single longitudinal section (Douglas, 1916, fig. 50) as a narrow gill apparently growing downward into the gill cavity. The secondary gills are merely short gills extending from the margin of the pileus part way to the stipe.

In diagram 4 we have a primary gill and two shorter ones superimposed. The line *A* cuts through the primary gill which extends from the stipe to the margin of the pileus, and its width is represented in diagram 5, *A*. Here we have the full width of the gill. The line *B*, diagram 4, cuts through the primary and one of the secondary gills, and the widths of the two gills are shown in diagram 5, *B*. The secondary gill through the region *B* is narrower than we find it at *C*. Here the primary and the secondary gill are of the same width. Similarly, we find the shortest gill narrower than the two preceding gills through *D*, but it becomes of equal width in the plane *E*.

Coprinus ephemerus is especially favorable material because of the small number of gills and the relatively late appearance of the secondary gills. Their development is entirely similar to that I have already described for *Coprinus micaceus*. In the tangential section shown in figure 81 we are near the margin, as shown in diagram 6 by the line *BB*. In this section we find a narrow gill. The second gill cavity from the left shown in figure 82 represents the left-end one in figure 81, and the third gill cavity represents the larger middle one in which the secondary or short gill appears to hang freely in figure 81. This short gill is not actually free through all its length as it would be if it were developing and growing down into the gill chamber, for in the succeeding parallel tangential section shown in figure 80, made toward the outer surface of the carpophore at a region indicated by the line *AA* in diagram 6, we have the four *Anlagen* of the gill cavities and the younger or marginal part of the secondary gill firmly joined to the fundamental tissue below. Notice how closely the two middle gill-cavity *Anlagen* lie to one another. The crowded condition is indicative of the development of a gill cavity in a space which formerly allowed only one. The tramal region recognizable between the *Anlagen* of the other gill cavities is much greater and wider, showing an undisturbed condition since no new cavities are forming.

It appears, then, that as the circumference of the young carpophore becomes greater the distance between the young lamellae at the margin becomes greater, and in the regions of the fundamental tissue and of the palisade cells between these primordia of the lamellae, further differentiation of these tissues occurs which results in the formation of a new tramal plate bounded by palisade cells, which thus form a new gill—the short or secondary gill. The old gill cavity is split by the new gill. The apparent suspension of the secondary gill is due to the growth of the inner margin of this gill



DIAGRAMS 7–12. Top view of a series of carpophores showing the development of a secondary gill at "*Z*" by the development of a secondary gill cavity at "*Y*." *e'* represents a region where the undifferentiated fundamental tissues of the stipe and pilear regions meet at the periphery of the carpophore; *f*, the increase in growth of the secondary gill.

toward the stipe (see diagram 4), giving this edge a curved outline. Diagrams 7 to 12 inclusive represent the development of a secondary gill. The letter *a* represents the undifferentiated region of the pilear structure, *b* the smooth palisade cells, *c* the oriented palisaded cells, *d* the young gills, and *e* the stipe region; *e'* represents a region where the undifferentiated fundamental tissue of the stipe and pilear regions, at the periphery of the carpophore, meet; *f* represents the increase in growth of the secondary gill.

This series of diagrams shows a carpophore increasing gradually in size. With growth, the distance between the lamellae at *I*, diagram 7, becomes greater, and more palisaded cells appear between the tramal elements as shown at *x* in diagram 8. A new center upon which the new palisade cells become oriented, at *y* in diagram 9, and a point *z* on the stipe fundament *e'*, become established with the differentiation of a new rudimentary trama, the hyphae of which are continuous between the stipe and the pilear regions. Continued growth upward into the pileus (diagram 10) makes the new

center *y* as fully developed as *x*, and subsequent development brings about the opening of the palisade cells *x* and *y*, forming a young secondary gill which divides the old gill cavity into two. The young gill is fixed at *z* because of the continuity of the tramal elements between the pileus and stipe hyphae in this region. Further development of the gill cavities increases the width of the primary and secondary gills represented by *f* in diagrams 11 and 12. Growth takes place peripherally to *z*, at *x* and *y*.

Very often these secondary gills break away from the fundamental tissue below them so that they remain somewhat narrower than the primary gills. It appears from these sections and from hundreds of similar ones that the secondary gills have a development identical with that of the primary gills except that their point of origin is at some distance from that of the primary gills.

The cells of the stipe resemble those of other species of Coprinus in that they are multinucleated. The globular cells on the surface of this button shown in figure 83, Plate XXXIV, disappear at a later stage, as shown in figure 84. The cells making up the trama also disappear up to and through the pileus, so that the hymenial surface alone appears in older buttons. This condition gives the pilear surface its fluted or corrugated appearance. Figures 91, 92, and 93 represent cross, longitudinal tangential, and longitudinal median sections of the older carpophores of *Coprinus stercorarius*. The cells covering the pileus of this species apparently plasmolyze readily and give the appearance of globular cells with an irregular dark-staining body in their interior. These are intermingled with cylindrical hyphal threads which also appear to be plasmolyzed. These cells give the scaly appearance of the mature pileus. I have observed carpophores with attenuated stems like those described by Brefeld. The pilei are much reduced and the stipes are very long, as shown in figure 88, Plate XXXV. The gills are merely narrow ridges as shown in figures 89 and 90, the latter of which represents a longitudinal tangential section. These forms are plainly abnormal. They may be found in material collected in the field among the belated specimens.

I have also studied sections of *Coprinus atramentarius* made from material collected in the field. Buttons in all stages were found in a cluster that appeared close to a group of plants already mature. These young plants were fixed in diluted Flemming's weak solution, and the stages studied agree with those described for *C. micaceus*. No annular gill cavities were observed. Figures 94 and 95 represent median and tangential longitudinal sections respectively. The young gill *Anlagen* in both cases are attached to the fundamental tissue of the stipe region. It is clear from the data given above that, in the species of Coprinus so far described, the development of the carpophore and the formation of the gills are practically the same, with but slight variations as to the time of formation of the stipe or of the pileus.

SUMMARY

1. Fresh buttons of *Agaricus campestris* fixed in the standard fixing agents show shrinkage and tearing of the fundamental tissue within 6 to 24 hours after fixation.

2. Fresh and dead carpophores of *A. campestris* studied show no primary annular gill cavity but a series of arches or gill cavities between each pair of gills with the trama tissue continuous with the hyphae of the pileus and of the stipe rudiments. There is no one general annular gill cavity but a number of interlamellar cavities similar to those found in the Coprinus species.

3. Spores of *Coprinus ephemerus* and *C. stercorarius* sown on dung or bean agar produce carpophores within 10 days after the inoculation is made.

4. The early stages in the development of the carpophore *Anlage* in these species is similar to that in *C. micaceus*. The early stages in the differentiation of the carpophore of these species are similar, up to the time of the development of the lamellae, to those of other types of agarics already described. The development of the lamellae of *C. ephemerus* and *C. stercorarius* is similar to that described by me for *C. micaceus*. No general annular gill cavity is formed.

5. The primordium of the hymenium arises as pockets of palisade cells with the ends of these cells pointing downward. As they increase in number they form a small arch, enclosing thus an interhyphal space between their free ends. This interhyphal space is the beginning of a gill cavity. The palisade cells forming the vertical walls of the arch are the rudiments of the adjacent hymenia of two gills. The vertical plate of hyphae between two adjacent arches constitutes the rudimentary trama, which is continuous with the hyphae of the stipe below and of the pilear fundament above. The young gill in its earliest stages of development is composed of the tramal cells together with the adjacent vertical walls of two arches.

6. Secondary or short gills arise in a manner similar to that of the primary gills. As the pilear rudiment increases in diameter, the distance between two adjacent rudiments of primary lamellae of the margin becomes greater and greater. A new pocket of palisade cells is intercalated at the margin of the pileus between two pockets of cells already formed and which are already open near the stipe. A cluster of new tramal hyphae is also formed which is fixed and continuous between the stipe and the pileus, as are the trama cells of the primary gills. As the palisade cells of the new pocket and the cells of the already formed adjacent pocket increase, two small arches are formed, separated by the newly formed trama and the adjacent hymenia. The trama and the adjacent hymenial surfaces constitute the short gill. This divides the former gill cavity into two.

7. The short gills are attached to the stipe fundament from the very beginning and do not grow downward between two old gills.

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LITERATURE CITED

Adams, J. F. 1918. Origin and development of the lamellae in *Schizophyllum commune*. Mem. Torrey Bot. Club **17**: 326–333. Pl. 9, figs. A, B.

Atkinson, G. F. 1906. The development of *Agaricus campestris*. Bot. Gaz. **42**: 241–264. Pls. 7–12.

——. 1914*a*. The development of *Agaricus arvensis* and *A. comtulus*. Amer. Jour. Bot. **1**: 3–22. Pls. 1, 2.

——. 1914*b*. The development of *Lepiota clypeolaria*. Ann. Myc. **12**: 346–356. Pls. 13–16.

——. 1914*c*. The development of *Amanitopsis vaginata*. Ann. Myc. **12**: 369–392. Pls. 17–19.

——. 1914*d*. The development of *Armillaria mellea*. Myc. Centralbl. **4**: 113–121. Pls. 1, 2.

——. 1915. Morphology and development of *Agaricus Rodmani*. Proc. Amer. Philos. Soc. **54**: 309–343. Pls. 7–13, figs. 1–8.

——. 1916*a*. Origin and development of the lamellae in Coprinus. Bot. Gaz. **61**: 89–130. Pls. 5–12, figs. 1–6.

——. 1916*b*. The development of *Lepiota cristata* and *L. seminuda*. Mem. N. Y. Bot. Gard. **6**: 209–228. Pls. 21–26.

Bonorden, H. F. 1851. Handbuch der allgemeinen Mycologie. Stuttgart.

Brefeld, O. 1877. Botanische Untersuchungen über Schimmelpilze. Heft 3. Basidiomyceten **1**: 1–135. Pls. 1–9.

Buller, A. H. R. 1909. Researches on fungi. London.

De Bary, A. 1866. Morphologie und Physiologie der Pilze, Flechten und Myxomyceten. Leipzig.

——. 1887. Comparative morphology and biology of the fungi, mycetozoa, and bacteria. (Eng. transl.) Oxford.

Douglas, Gertrude E. 1916. A study of development in the genus Cortinarius. Amer. Jour. Bot. **3**: 319–335. Pls. 8–13.

Duggar, B. M. 1915. Mushroom growing. New York.

Dutrochet, H. J. 1837. Mémoires pour servir á l'histoire anatomique et physiologique des végétaux et des animaux **2** : 370–377. Pl. 22.

Fayod, V. 1889. Prodrome d'une histoire naturelle des Agaricinés. Ann. Sci. Nat. Bot. VII, **9**: 181–411. Pls. 6, 7.

Harper, E. T. 1913. Species of Pholiota and Stropharia in the region of the Great Lakes. Trans. Wis. Acad. Sci., Arts, Lett. **17**^{2}: 1011–1026. Pls. 59–67.

——. 1914. Species of Hypholoma in the region of the Great Lakes. Trans. Wis. Acad. Sci., Arts., Lett. **17**^{2}: 1142–1164. Pls. 72–84.

Hartig, R. 1874. Wichtige Krankheiten der Waldbäume. Berlin.

Hoffmann, H. 1856. Die Pollinarien und Spermatien von Agaricus. Bot. Zeit. **14**: 137–148, 153–163. Pl. 5.

——. 1860. Beiträge zur Entwickelungsgeschichte und Anatomie der Agaricinen. Bot. Zeit. **18**: 389–396, 397–404. Pls. 13, 14.

——. 1861. Icones analyticae fungorum. Giessen.

Levine, M. 1913. Studies in the cytology of the Hymenomycetes, especially the Boleti. Bull. Torrey Bot. Club **40**: 137–181. Pls. 4–8.

——. 1914. The origin and development of the lamellae in *Coprinus micaceus*. Amer. Jour. Bot. **1**: 343–356. Pls. 39, 40.

Nees von Esenbeck, C. G. 1816. Das System der Pilze und Schwämme. Würzburg.

Sawyer, W. H. 1917*a*. Development of some species of Pholiota. Bot. Gaz. **64**: 206–229. Pls. 16–20.

——. 1917*b*. The development of *Cortinarius pholideus*. Amer. Jour. Bot. **4**: 520–532. Pls. 28, 29.

Schmitz, J. 1842. Mycologische Beobachtungen als Beiträge zur Lebens- und Entwickelungsgeschichte einiger Schwämme aus der Klasse der Gasteromyceten und Hymenomyceten. Linn. **16**: 141–215. Pls. 6, 7.

Trog, J. B. 1837. Ueber das Wachsthum der Schwämme. Flora **20**: 609–618.

Walker, Leva B. 1919. Development of *Pluteus admirabilis* and *Tubaria furfuracea*. Bot. Gaz. **68**: 1–21. Pls. 1–5, figs. 1–8.

DESCRIPTION OF PLATES

PLATE XXVIII

Buttons of *Agaricus campestris* vars. *Bohemia* and *Columbia*.

FIGS. 1–16. Stages in development of fresh carpophores showing the young gills attached to the fundamental tissue below the pilear region. Figs. 4, 9, 13, 14, and 15 are tangential sections, showing no annular gill cavity and the small gills still attached to the tissue which will develop into the annulus. Photographed 5 to 20 minutes after free-hand sections were made. × 2½.

FIGS. 17–22. Young buttons which have turned brown and died 48 to 96 hours after their appearance on the substratum. Longitudinal sections show no annular gill cavities, but the gill *Anlagen* are attached to the fundamental tissue below them. Photographs made from buttons 5 to 20 minutes after being sectioned free-hand. × 3.

FIGS. 23–28. Dead buttons of the same stage of development after having been in a chrom-acetic solution for 24 hours show no annular gill cavities; the rifts below the young gills were caused by cutting the fluid-soaked buttons. × 3.

FIGS. 29–34. Fresh, actively growing buttons sectioned longitudinally after being exposed to Flemming's weaker solution for 48 hours; the so-called annular gill cavities appear and the young gills appear to be growing downward into them. Photographs were made from 5 to 20 minutes after removal from fluid. × 3.

FIGS. 35–41. Young, actively growing buttons which were subjected to a chrom-acetic solution for 2 hours show exceedingly large annular gill cavities below the developing lamellae. Figures 35, 38, and 40 are tangential sections showing the so-called annular gill cavity as a wide slit due to the shrinkage of the tissue caused by the fixing agent. × 3.

FIGS. 42–44. Actively growing buttons, after being fixed in Flemming's strong solution 6 to 18 hours, show shrinkage which causes the development of the annular gill cavity.

FIGS. 45–51. Fresh buttons after having been fixed for from 6 to 18 hours: figure 45, in Gilson's solution; figure 46, in Juel's; figure 47, in Bouin's; figure 48, in Merkel's; figure 49, in Kaiser's; figure 50, in picro-acetic; figure 51, in Carnoy's. All × 3. All show tremendous shrinkage, which accounts for the appearance of the annular gill cavity.

PLATE XXIX

The microphotographs on this and the following plates were made with the aid of the Zeiss microphotographic apparatus and Leitz objectives nos. 3 and 6, and oculars nos. 1, 3, and 4.

Figures 52 to 62 represent sections of *Agaricus campestris*.

FIG. 52. Longitudinal tangential section of a young dead carpophore, showing the trama of the young lamellae continuous with the fundamental tissue below the pileus and forming a series of interlamellar chambers. Ocular 1, objective 3, bellows 20 cm.

FIG. 53. A dead button slightly older than the one shown in the previous figure, cut tangentially to the long axis of the carpophore; it shows wide tramal tissue and the hymenia. The trama is continuous with the fundamental tissue below. Ocular 3, objective 4, bellows 20 cm.

FIGS. 54, 55. Longitudinal tangential and median sections of a young dead button slightly older than the one shown in figure 53. The hymenial surface is beginning to

disintegrate and the fundamental tissue to collapse, as shown in figure 54. Continuity of the hyphae between the trama and the fundamental tissue may still be seen in both figures. Ocular 4, objective 3, bellows 20 cm.

FIGS. 56, 57. Sections of a young dead carpophore 96 hours old after it had failed to show signs of further development. FIG. 56. Longitudinal tangential section near the margin of the pilear region, showing the trama as more heavily stained columns of tissue continuous with the less deeply stained hyphae of the fundamental tissue below. FIG. 57. Longitudinal tangential section near the stipe, showing the tearing away of the trama of the young gills from the fundamental tissue, the annulus, resulting in the furrows on its surface at *A*. Ocular 4, objective 3, bellows 20 cm.

PLATE XXX

FIG. 58. A longitudinal median section of the same carpophore shown in figures 56 and 57, showing the young gills torn away from the fundamental tissue. Ocular 4, objective 3, bellows 20 cm.

FIGS. 59–61. Longitudinal median sections of young carpophores which had turned brown after being on the bed 48 to 72 hours and having failed to show signs of growth, showing the trama of the young gills continuous with the fundamental tissue; the involute margin is not strongly developed. Ocular 4 (figure 61, ocular 1), objective 3, bellows 20 cm.

PLATE XXXI

FIG. 62. Longitudinal median section of abnormal button showing a densely staining, bowl-shaped central structure comparable to Fayod's *couche piléogène*. Ocular 4, Spencer objective 32 mm., bellows 20 cm.

FIG. 63. Enlargement of part of figure 62, showing the structure of the *couche piléogène*. Ocular 1, objective 3, bellows 20 cm.

FIG. 64. Longitudinal median section of abnormal button slightly older, showing poroid gills to the right and left of the *couche piléogène*. Ocular 4, Spencer objective 32 mm., bellows 20 cm.

FIG. 65. Enlargement of figure 64, showing more clearly the nature of the poroid gills. Ocular 3, objective 3, bellows 20 cm.

PLATE XXXII

Figures 66 to 84 inclusive represent *Coprinus ephemerus*.

FIG. 66. A pure culture grown in Petri dishes on dung agar, showing sclerotia and radiating rhizomorphs on which minute carpophores are beginning to appear.

FIG. 67. A later stage, with much branched and anastomosing mycelium covered with great numbers of young carpophores.

FIG. 68. A pure culture, showing an abundance of young carpophores near the margin of the dish; in the center, a mass of sclerotia surrounded by a weft of aerial hyphae.

FIGS. 69, 70. Microphotographs of a young carpophore. FIG. 69. Longitudinal tangential section, showing four pockets of palisade cells with three groups of intervening tramal tissue continuous between the pilear and stipe regions shown at *A*. FIG. 70. Median longitudinal section of the same carpophore; the cluster of deeply staining cells to the left represents a radial view through a pocket of palisade cells. The less deeply stained cluster of cells is a section through the trama. Ocular 3, objective 6, bellows 40 cm.

PLATE XXXIII

FIGS. 71–73. A series of sections of a young carpophore grown on agar. FIG. 71. Longitudinal median section of a young carpophore through two clusters of oppositely

placed pockets of palisade cells. FIG. 72. Longitudinal tangential section showing three clusters of oriented palisade cells at *A*. FIG. 73. Tangential section showing four clusters of palisade cells and three intervening groups of tramal hyphae at *C*. Ocular 3, objective 6, bellows 40 cm.

FIGS. 74–76. A series of sections of a carpophore slightly older than the one shown in previous sections. FIG. 74. Longitudinal median section through two oppositely placed pockets of palisade cells. FIG. 75. Longitudinal tangential section showing four pockets indicated by *A*, in which the second pocket from the left is beginning to open, forming an interlamellar space. FIG. 76. Longitudinal tangential section through the margin of the carpophore, showing three pockets of palisade cells (*A*) not yet open. Ocular 3, objective 6, bellows 40 cm.

FIGS. 77–79. A series of sections of a carpophore still older. FIG. 77. Longitudinal tangential section near the margin of the pilear region, showing two pockets of palisade hyphae. FIG. 78. Longitudinal tangential section near the stipe, showing two well-developed interlamellar cavities; two pockets of palisade cells on either side at the margin. FIG. 79. Longitudinal tangential section still nearer the stipe, showing four distinct interlamellar cavities and a marginal pocket of palisade cells to the left. Ocular 3, objective 6, bellows 40 cm.

FIGS. 80–82. A series of sections of a young carpophore with secondary gills. FIG. 80. Longitudinal section near the margin, showing four pockets of palisade cells; the middle two lie close together, indicating the presence of a secondary gill between them, as shown in figure 81 which is a longitudinal tangential section nearer the stipe showing a short gill which in more eccentric sections is attached to the fundamental tissue below it. FIG. 82. A section still nearer the median, showing the disappearance of the short gill and the presence of four gill cavities and three young lamellae continuous with the fundamental tissue below them. Ocular 4, objective 3, bellows 50 cm.

PLATE XXXIV

FIG. 83. Longitudinal section near the stipe, showing attachment of mature gills to the fundamental tissue. Ocular 3, objective 3, bellows 45 cm.

FIG. 84. Cross section of a carpophore showing the disintegration of the fundamental tissue between the gills and stipe; no spores are present at the margins of the gills. The globular cells seen on the surface of the pileus in earlier stages have disappeared, leaving a fluted surface. Ocular 3, objective 6, bellows 20 cm.

FIG. 91. Portion of a cross section of the pileus of *Coprinus stercorarius*, showing the attachment of the primary gills to the stipe.

FIG. 92. Portion of a longitudinal tangential section of a carpophore of the same age, showing the young gills still attached to the fundamental tissue; it shows clearly the type of cells covering the pileus which give it its scaly appearance.

FIG. 93. Portion of a longitudinal median section. Ocular 1, objective 3, bellows 20–25 cm. for figures 91–93.

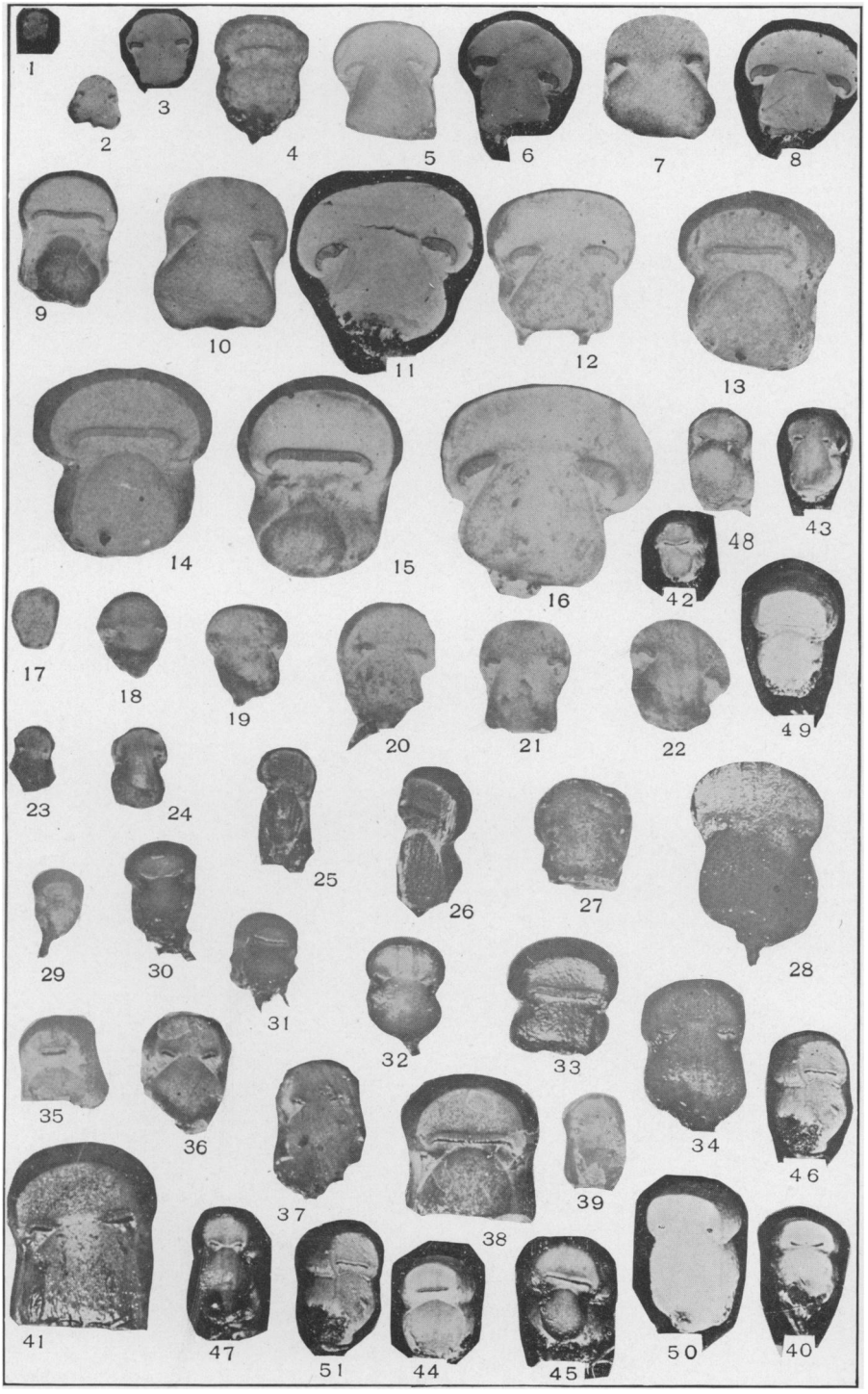
PLATE XXXV

FIG. 85. Early stages in the development of a carpophore of *C. stercorarius* grown on agar. The weft of hyphae arising from a number of cells below the surface of the agar, shown at *A*. Ocular 4, objective 3, bellows 20 cm.

FIG. 86. Early stages in the development of a carpophore of *C. stercorarius* on the stipe of an older button. *B*, a portion of the cross section of the older stipe. Ocular 4, objective 3, bellows 20 cm.

FIG. 87. Early stages in differentiation of a young carpophore of *C. stercorarius*, showing the development of the pilear mass. Ocular 4, objective 3, bellows 20 cm.

FIG. 88. Longitudinal median section of young carpophore with long, attenuated



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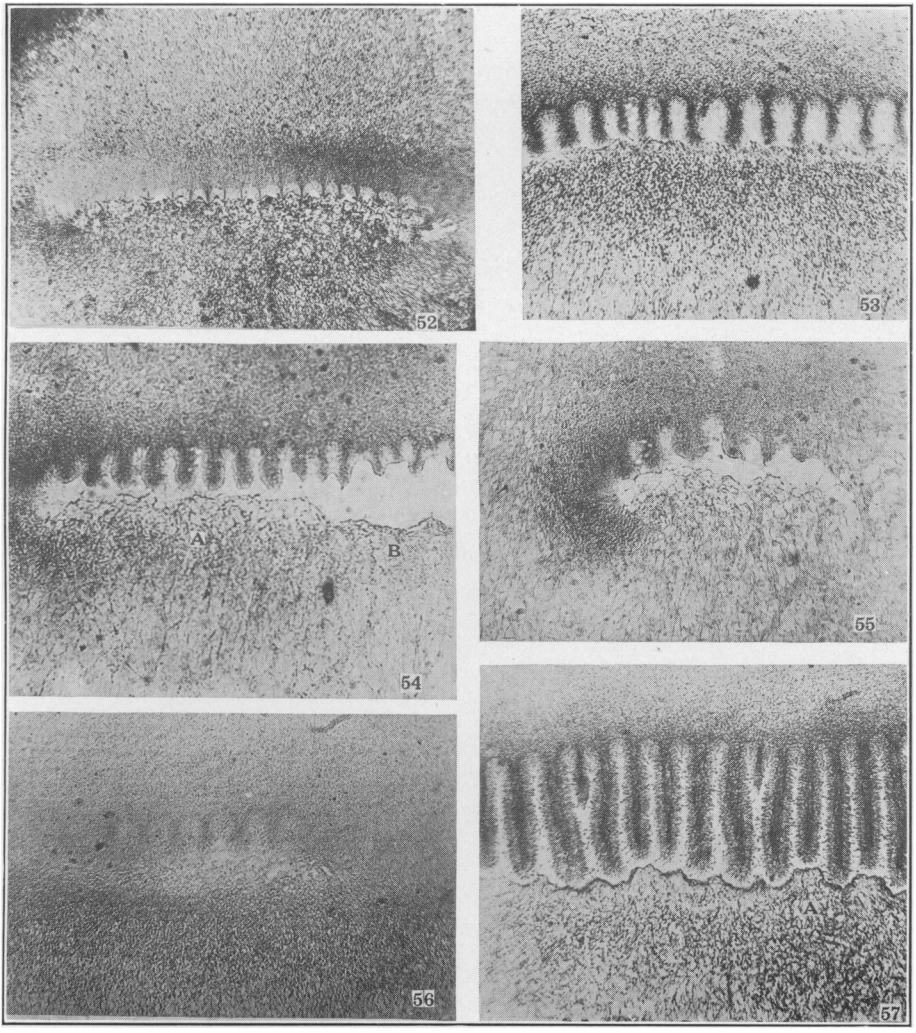

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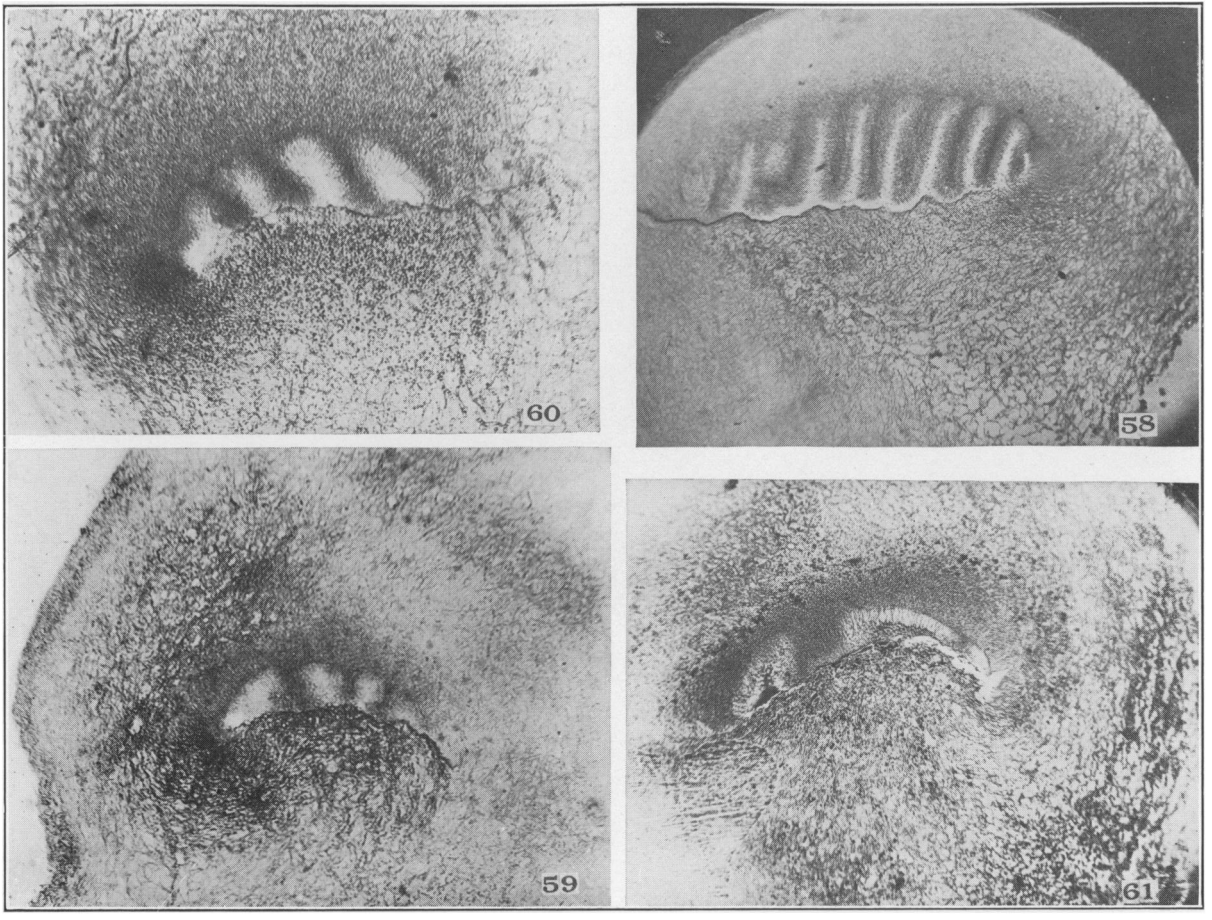

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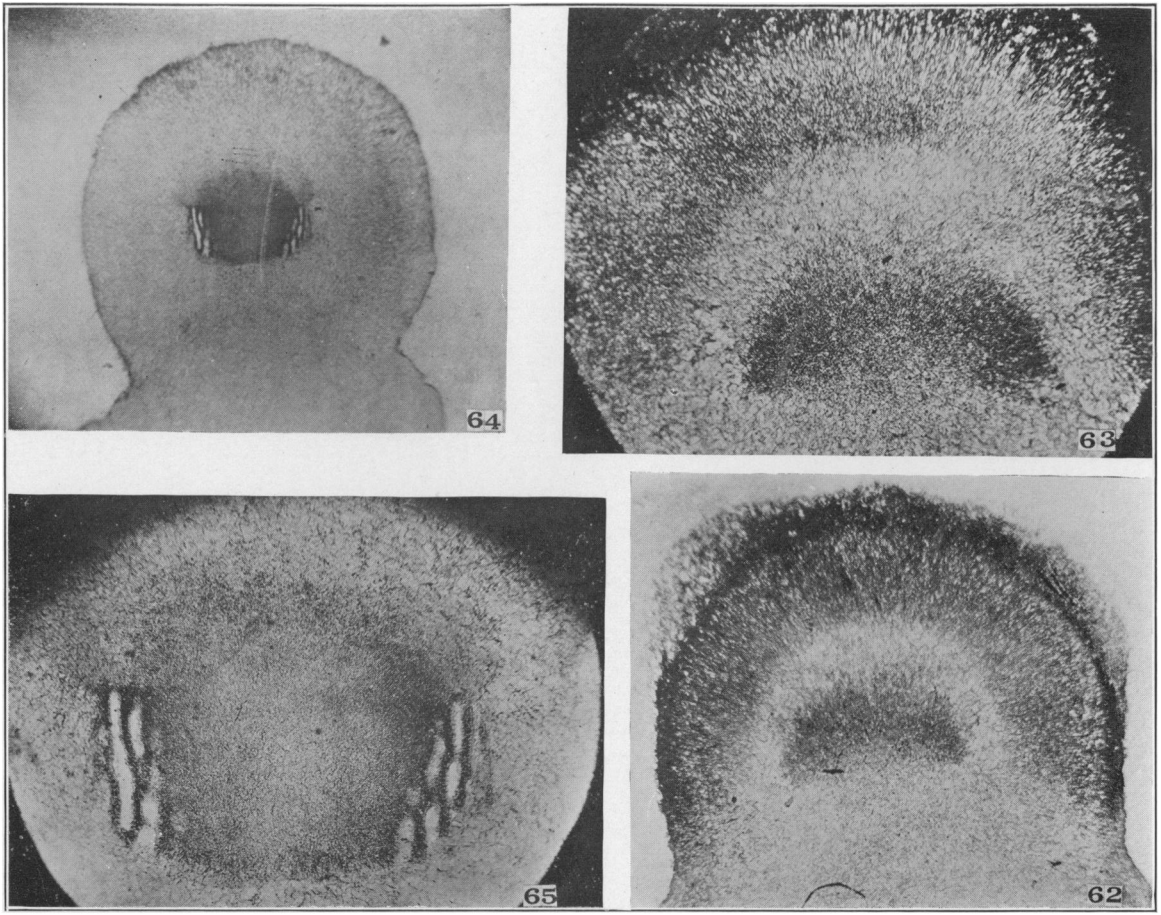

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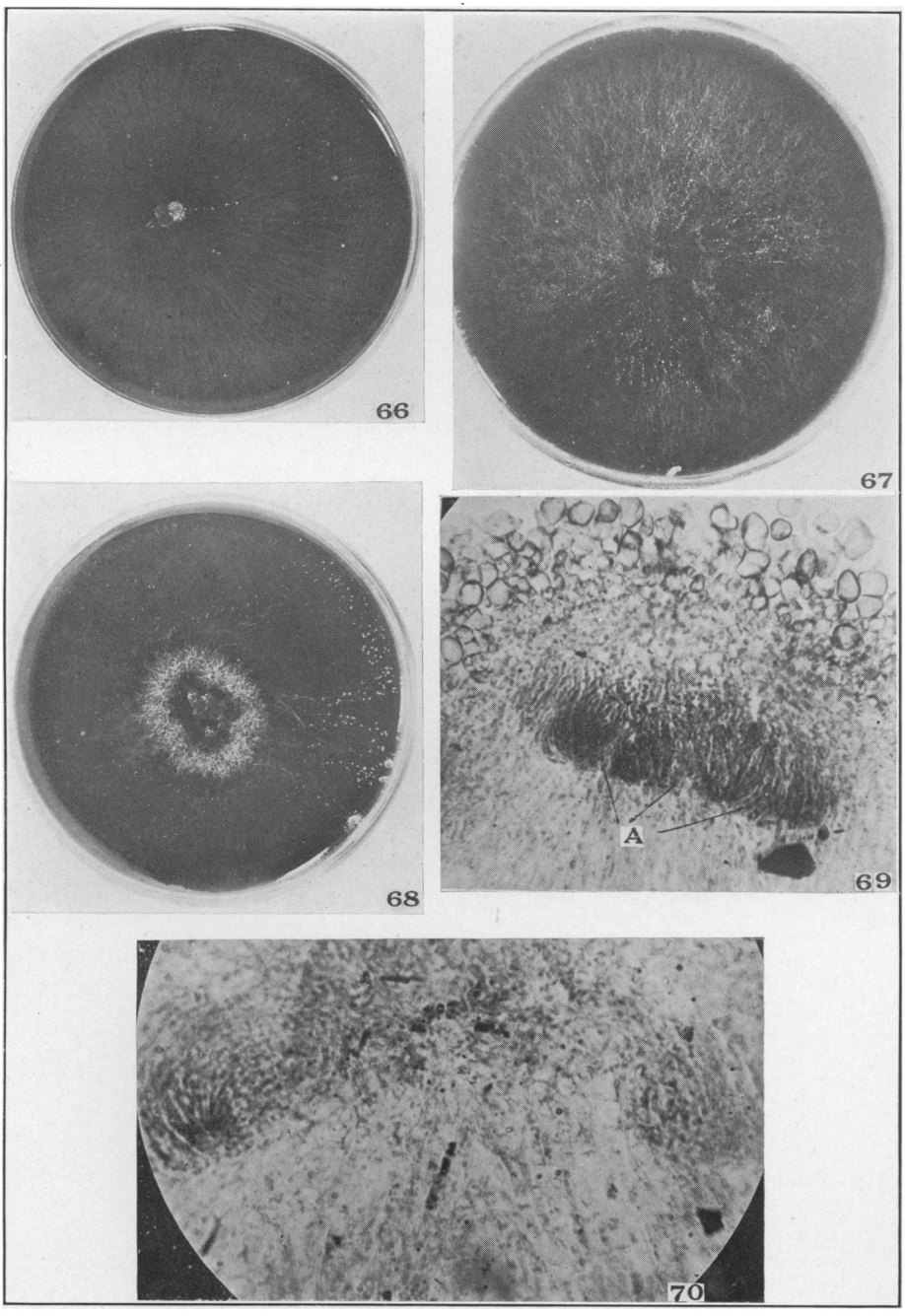

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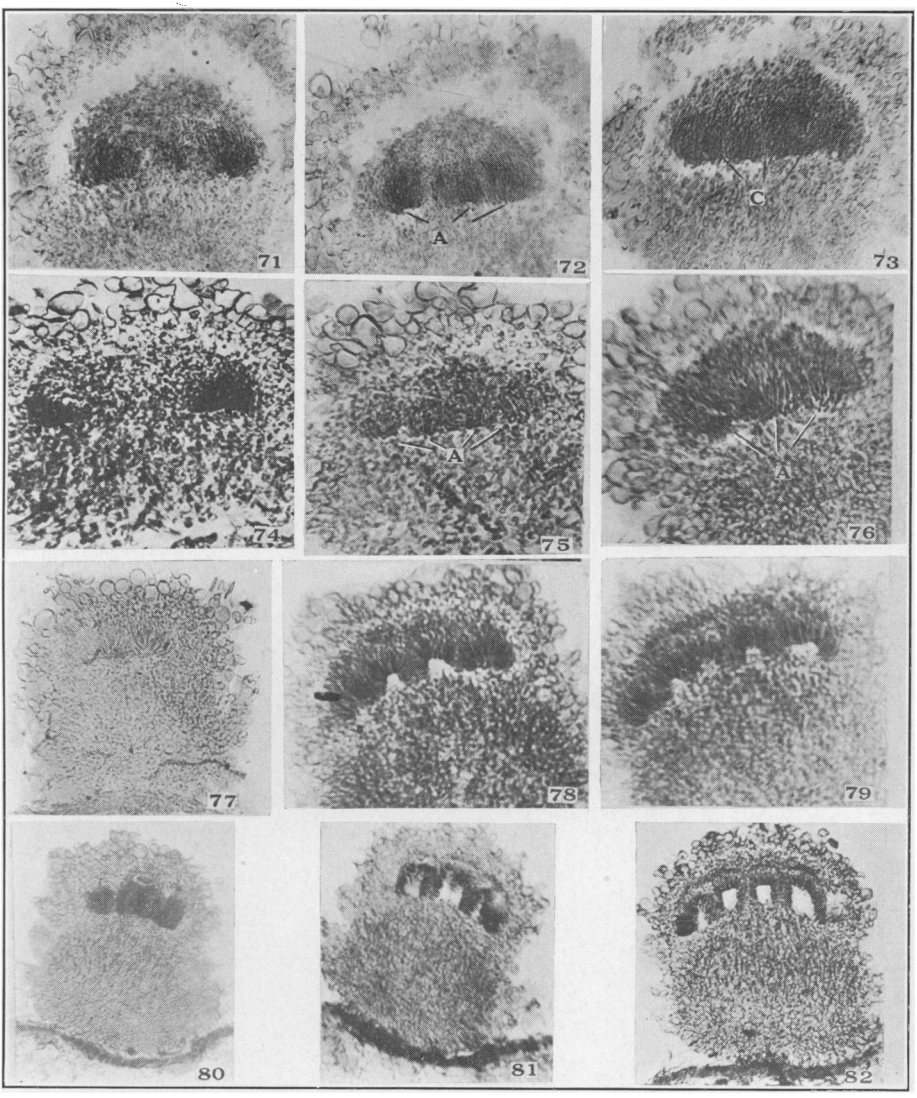

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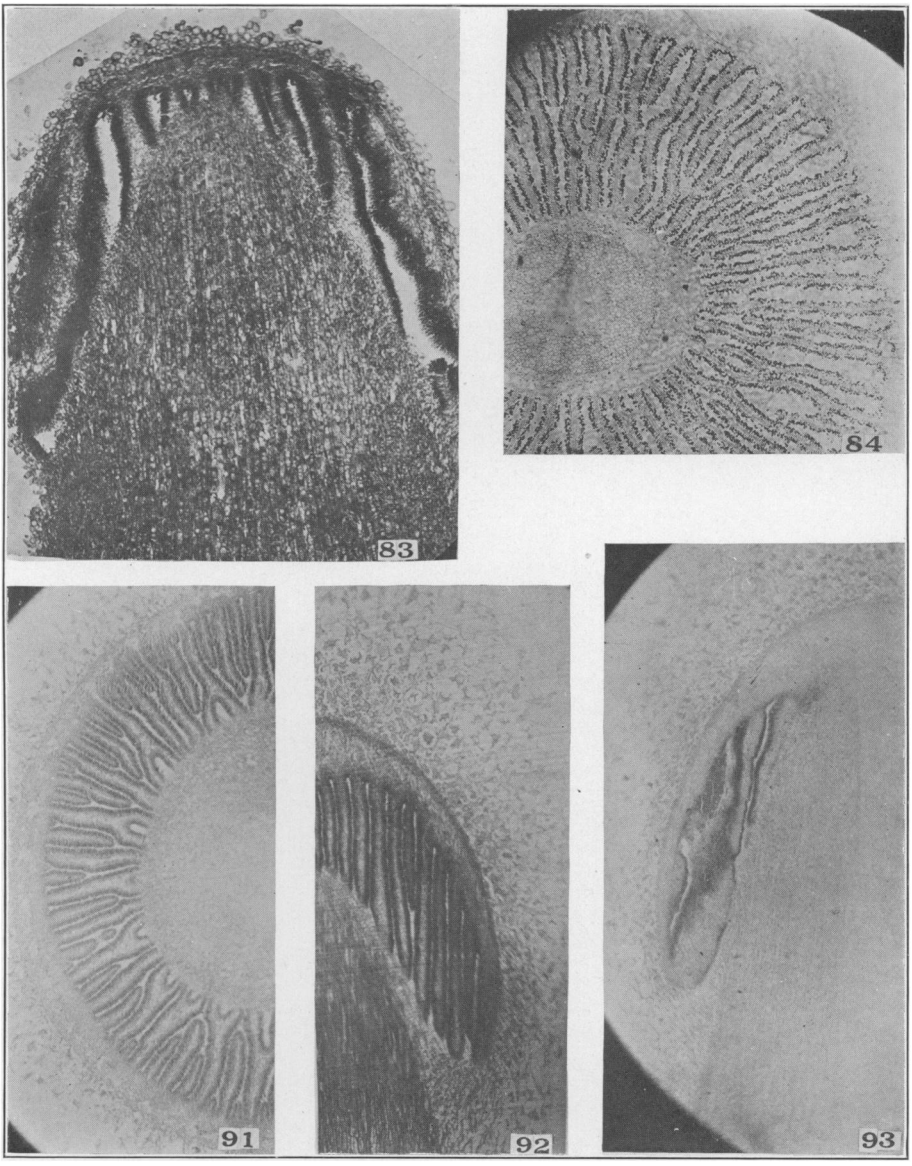

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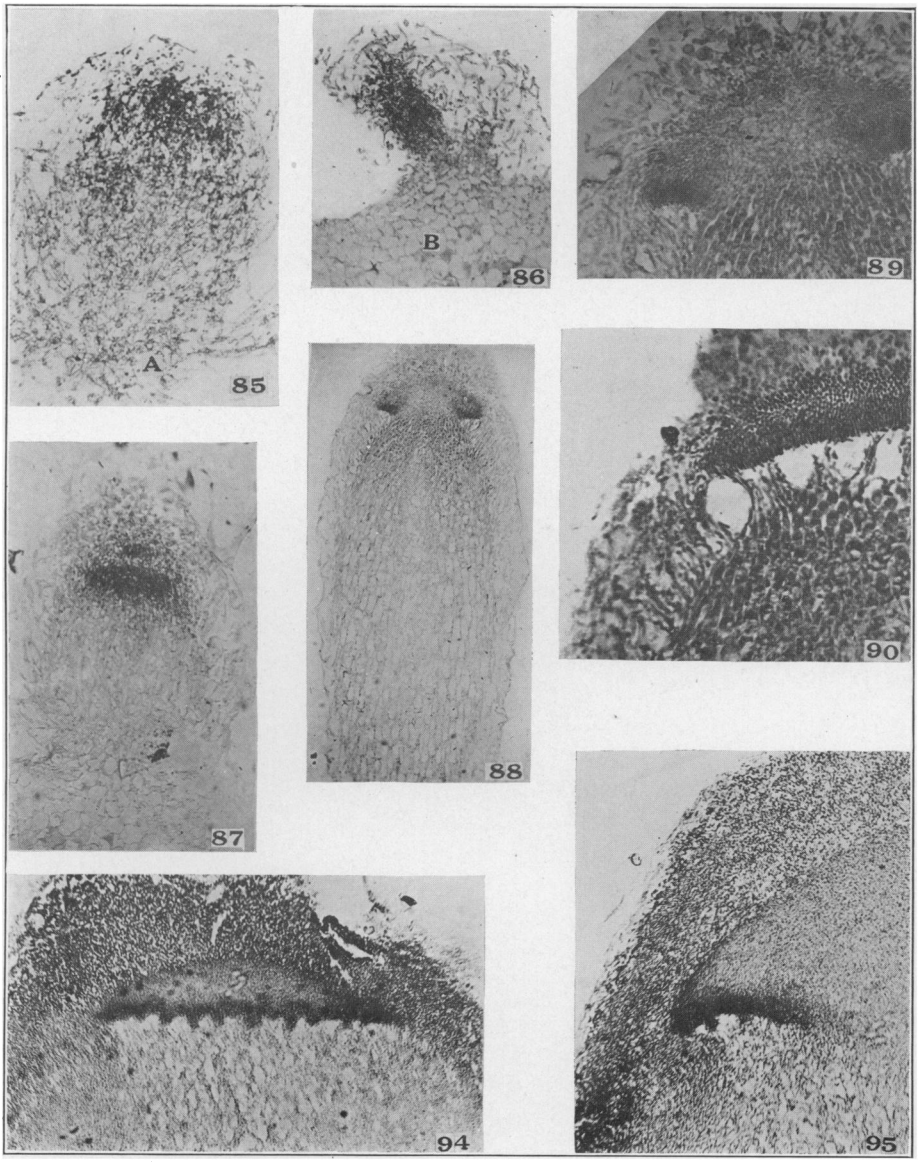

LEVINE: DEVELOPMENT OF LAMELLAE

stipe and a pilear region with no true lamellae forming. Ocular 3, objective 3, bellows 20 cm.

FIG. 89. An enlargement of a portion of the pileus shown in figure 88, showing a smooth hymenial surface. Ocular 3, objective 6, bellows 20 cm.

FIG. 90. Longitudinal tangential section of a similar button, showing a smooth hymenial surface with some of the fundamental tissue hyphae of the stipe continuous with the hyphae of the pileus. Ocular 3, objective 6, bellows 20 cm.

FIGS. 94, 95. Longitudinal tangential (fig. 94) and median (fig. 95) sections of a young button of *C. atramentarius*, showing the trama of the young gills continuous with the fundamental tissue of the stipe below. Ocular 1, objective 3, bellows 20 cm.